THE EFFECTS OF DIISOPROPYLFLUOROPHOSPHATE ON SPATIAL FREQUENCY RESPONSIVITY IN THE CAT VISUAL SYSTEM

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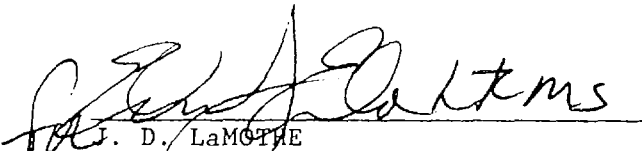originator.

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Visual-evoked responses to counterphased gratings were recorded from area 17 of cat visual cortex before and after diisopropylfluorophosphate (DFP) admini- stration. DFP produced effects similar to those obtained following physostig- mine sulfate administration, in that responses to low spatial frequencies were preferentially reduced. The time course of the effects was quite different for the two types of drugs, and for high doses of DFP responses to all spatial frequencies were approximately uniformly depressed or abolished.

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The effects of diisopropylfluorophosphate on spatial frequency responsivity in the cat visual system

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Visual-evoked responses to counterphased gratings were recorded from area 17 of cat visual cortex before and after diisopropylfluorophosphate (DFP) administration. DFP produced effects similar to those obtained following physostigmine sulfate administration, in that responses to low spatial frequencies were preferentially reduced. The time course of the effects was quite different for the two types of drugs, and for high doses of DFP responses to all spatial frequencies were approximately uniformly depressed or abolished.

Cholinergic influences have been found at various stages of processing within the primary visual pathway. In the cat, indirect evidence for cholinergic neurons has been found in retina[4], lateral geniculate nucleus[2,8], and visual cortex[7]. Recently, we have shown that the carbamate physostigmine sulfate preferentially reduces the cortical visual-evoked response (VER) to low spatial frequencies while minimally affecting the response to high spatial frequencies[3]. This non-uniform reduction in VER amplitude was considered cholinergic in nature since it could be reversed by the muscarinic antagonist, atropine sulfate.

To further investigate the cholinergic nature of the selective visual loss, we studied the effects of diisopropylfluorophosphate (DFP) on the cortical VER. DFP, an organophosphate, irreversibly binds acetylcholinesterase (AChE) thus preventing the hydrolysis of acetylcholine (ACh) at synaptic sites. Besides the anticholinesterase action of DFP and physostigmine, the two drugs may have distinctively different actions on the postsynaptic membrane[1,6]. In this report, we substantiate the original observations concerning cholinergic influences on the VER. Also, we show dose- and time-related effects following organophosphate administration.

Adult cats (2.3–4.0 kg) were anesthetized with halothane in a 3:1 mixture of nitrous oxide and carbogen. In addition, areas of surgical incision were anesthetized with lidocaine. Prior to physiological recording, the halothane was removed from the gas mixture. The trachea, one femoral artery and the two saphenous veins were cannulated. In order to reduce eye movements, the two sympathetic nerve trunks were cut and the animal was paralyzed by intravenous infusion of 30 mg/kg/h of gallamine triethiodide in an isotonic glucose solution. The animals were artificially ventilated and the end tidal CO_2 was maintained near 4% by adjusting the stroke volume of the respirator. Head position was stabilized in a stereotaxic head-holder and the core temperature was maintained near 37 °C.

A primary systemic effect of AChE-binding is stimulated secretory activity. Altered airway resistance could cause hypoxia and confound any changes in the VER. Therefore, blood gases and lung resistance were measured periodically and heart rate, blood pressure and EEG were monitored continuously throughout the experiments. If any of these measurements were abnormal, the VER recordings were discontinued until normality could be restored either by adjusting ventilation stroke volume and/or

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pre DFP   **spatial frequency**   **post DFP**
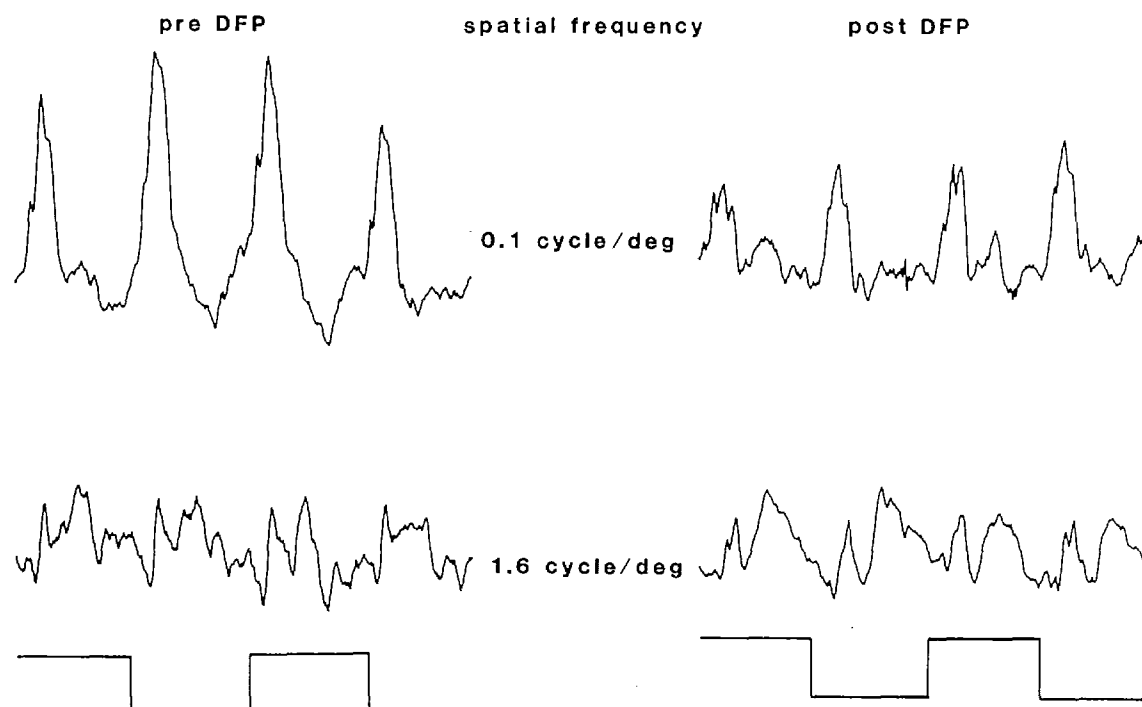

0.1 cycle/deg

1.6 cycle/deg

Fig. 1. VER averages (120 s collection period with a 1-ms sampling interval) for two spatial frequencies from a single experiment (cat 19). Histograms on the left are baseline VERs. VERs on the right were obtained during the first collection period immediately following 5 mg/kg DFP given i.v. over a 1-min period. The bottom row depicts the 2 Hz square wave alternation of the grating pattern. All response averages were collected with grating contrast of 0.40 $(L_{max} - L_{min})/(L_{max} + L_{min})$, where L is luminance.

by aspirating the airway. In practice, these corrective measures were seldom needed.

Atropine sulfate (1%) and phenylephrine hydrochloride (10%) were administered topically to the eye providing cycloplegia and retraction of the nictitating membrane. Contact lenses having 3 mm artificial pupils were fitted to the eyes to maintain the corneae and to reduce optical aberrations. Refractive errors were corrected with auxillary lenses which also focused the eyes to a cathode ray tube (CRT) which subtended a visual angle of $50° \times 42°$ at the viewing distance of 12.7 cm. For all recordings, only one eye was visually stimulated and the other eye occluded.

VERs were recorded from bone screws over visual (area 17) and parietal cortex. Square wave luminance gratings were generated on the CRT and phase alternated in square wave fashion at 2 Hz. Six spatial frequencies, each having a mean luminance of 82 cd/m^2, were presented in quasirandom fashion under computer control. Each frequency was presented for 10 s followed by a 1-s equivalent uniform luminance exposure. This continued until cumulative response averages of 120 s (twelve 10-s collection peri-
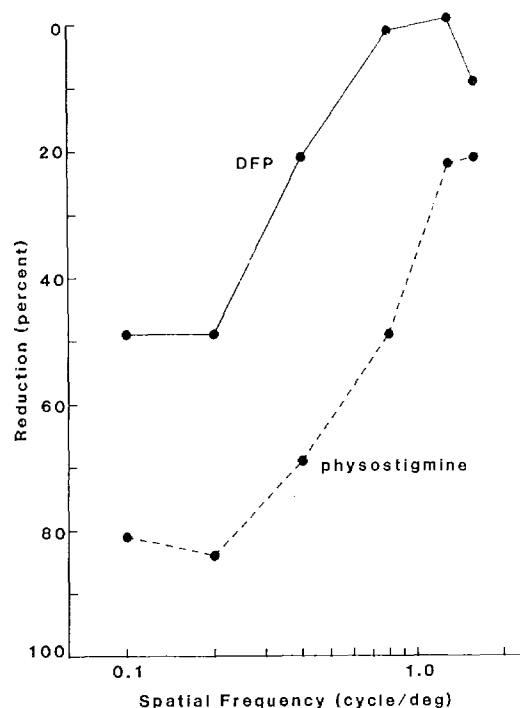

Fig. 2. Averaged data showing VER reduction for different spatial frequencies from 5 cats receiving 4.0 mg/kg DFP and 5 cats receiving 0.5 mg/kg physostigmine sulfate (dashed line). Grating contrast was 0.40.

ods) for each frequency were obtained. Our response measure was the sum of the amplitudes of the first five even harmonics of the fundamental (2 Hz; signal) less the sum of the first five odd harmonics (noise)[3,9]. Although square wave gratings were used, we use the term 'spatial frequency' to refer to the fundamental frequency. We showed previously that the results with square and sine wave gratings were essentially the same, in that we observed a preferential reduction at low spatial frequency with either grating type[3].

Fig. 1 shows averaged VERs collected prior to and following i.v. administration of DFP. A response peak occurs following each phase reversal of the grating, and for a 1-s epoch, four primary response peaks are seen. A secondary response peak was often pres-

ent and became more pronounced with increasing spatial frequency[3,9]. Following DFP administration, response amplitudes were reduced more at low spatial frequencies than at high spatial frequencies. For the histograms shown in Fig. 1, the high spatial frequency response was enhanced slightly following DFP and the increase in response appears to reflect the dominance of the secondary peak.

Fig. 2 shows the average reduction in VER amplitude obtained from 5 cats given 4.0 mg/kg DFP. This dose level caused an 83% average reduction in blood AChE measured 5 min following the dose. VER baselines were established by averaging the response amplitudes obtained from 4 or 5 repetitions of the 13.2-min stimulus sequence. For comparison, the average reduction in VER amplitude for 5 additional
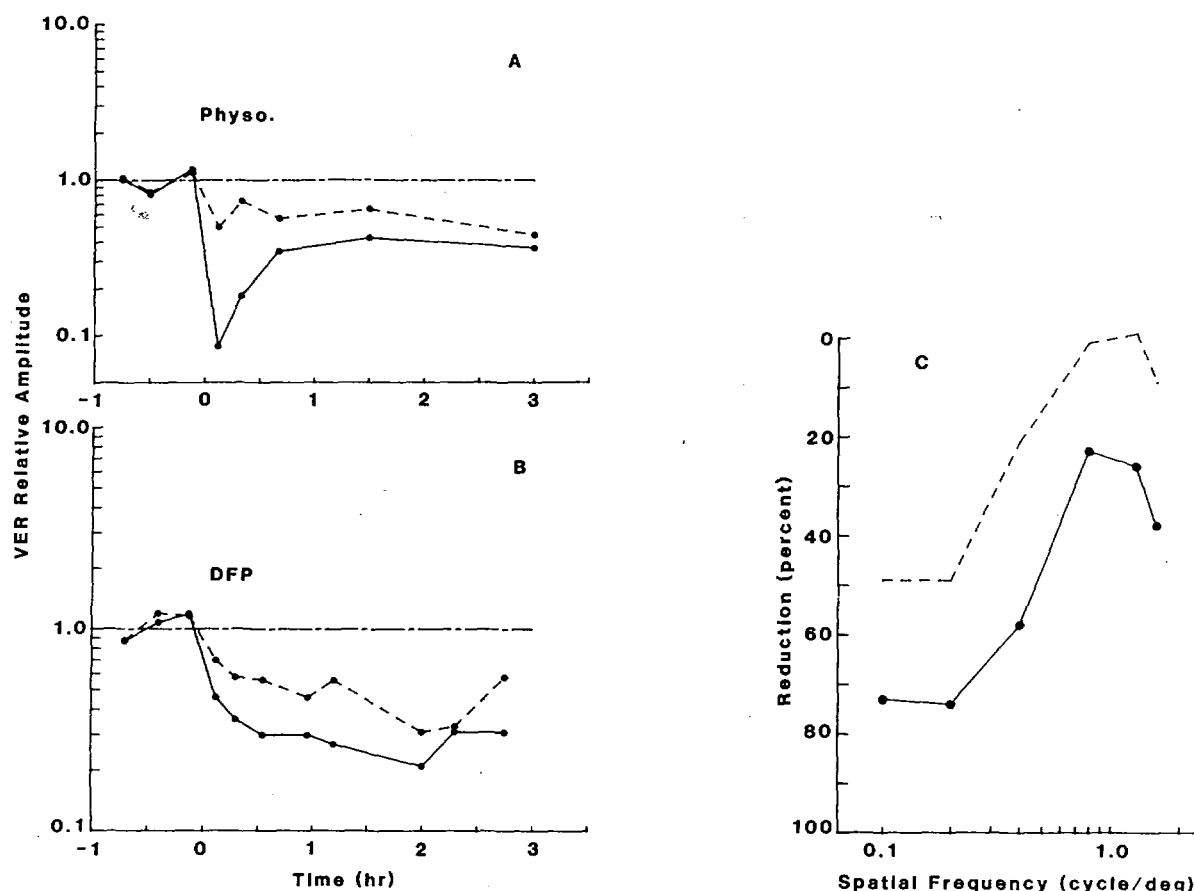

Fig. 3. Averaged VER amplitudes to the two lowest spatial frequencies (0.1 and 0.2 cycle/degree; solid lines) and to the two highest spatial frequencies (1.3 and 1.6 cycles/degree; dashed line) as a function of time before and following 0.5 mg/kg of physostigmine (A) and 4 mg/kg of DFP (B). The average of the baseline responses to both the low and high spatial frequencies was set equal to 1.0 (broken line). Data are from cats 11 (A) and 23 (B). C: average of the maximum reduction in VER amplitude for each spatial frequency following 4.0 mg/kg DFP for the same DFP cats as in Fig. 2. The DFP data from Fig. 2 showing initial reduction are replotted (dashed curve). Grating contrast was 0.40.

cats each receiving 0.5 mg/kg physostigmine sulfate also is shown as the dashed curve. For the physostigmine cats, blood AChE was reduced by an average of 46% at 5 min post-administration.

The results discussed thus far represent the effects observed immediately following drug administration, i.e. the effects occurring during the first 13.2 min. For physostigmine, the initial reduction in VER amplitude represents the maximum reduction since we typically observed the start of recovery during the second collection period (Fig. 3A). However, for DFP the maximum response reduction did not occur until approximately 2 h following the 4.0 mg/kg dose (Fig. 3B). In Fig. 3A and B, responses to low (solid line) and high spatial frequencies (dashed line) are plotted as a function of time following drug administration. The two curves shown in both Fig. 3A and B remain separate for the 3 h tracking period, indicating that the lower spatial frequency responses remained differentially reduced. Fig. 3C shows the average of the maximum reduction in VER amplitudes as a function of spatial frequency for the 5 cats receiving 4 mg/kg of DFP. Also plotted (dashed line) are the data from Fig. 2 showing the initial reduction.

Fig. 4 shows relative VER signal for the different spatial frequencies following 3 different doses of DFP. The graded effect is only apparent at moderate dose levels. At the highest dose (4.0 mg/kg; cumulative dose of 7.0 mg/kg), the VER essentially is abolished at all spatial frequencies. Additional experiments in two other cats following the same dose regimen showed similar results. Although VERs were not completely abolished, the graded effect was no longer apparent at the highest dose level where responses at all spatial frequencies were approximately uniformly reduced.

It could be argued that the observed preferential reduction at low spatial frequency might be due to our measurement technique. For gratings of equal contrast, as used in these experiments, the response magnitude at high spatial frequencies was always smaller than the response at low frequencies prior to drug administration. If responses following drug administration were all reduced to some noise level, measuring percent reduction would provide artifactual information. This logic assumes our estimate of noise is inappropriate. We feel that this is not the case for two reasons: (a) all of the 5 physostigmine
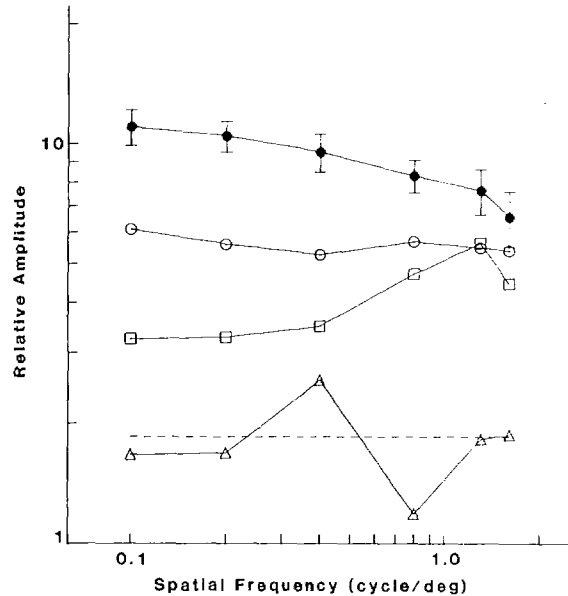

Fig. 4. Relative amplitudes of the VER signal (sum of the 5 even harmonics of the 2 Hz phase alternation; see text) for different spatial frequencies from cat 21. Filled circles represent the average of 5 baseline measures. Bars show ± 1 S.D. In ascending order, doses of 1 mg/kg (open circles), 2 mg/kg (open squares) and 4 mg/kg (open triangles) of DFP were given 2 h apart. Each point represents the average of 5 measurements except for the last dose which is the average of 3 measurements. The dashed line represents the amplitude of the average noise level (under all conditions) which is estimated by the sum of the 5 odd harmonics. For this cat, the amount of noise was independent of spatial frequency. Grating contrast was 0.40.

experiments (at 0.5 mg/kg dose level) and 2 of the 5 DFP experiments (at an initial dose of 4 mg/kg) show that the amplitudes of the VERs to high spatial frequencies were larger than the responses to low spatial frequencies following drug administration; and (b) in 2 of the physostigmine and 3 of the DFP experiments, the high spatial frequency responses were enhanced following administration. Therefore, these data provide strong support for a specific non-uniformity in spatial frequency responsivity following AChE inhibition.

Whether the observed effect is due to excessive ACh accumulation or to secondary factors mediating the primary effect is not clear. Harding et al.[3] showed that atropine sulfate reverses the visual loss following physostigmine administration, and subsequent studies show a similar reversal following DFP[5]. Atropine reversal could suggest a simple cholinergic-mediated effect due to ACh accumulation; however, recent findings[5] show that recovery of the VER can occur

over time without concomitant recovery in AChE activity. Also, we have not demonstrated a strong correlation between the magnitude of the VER reduction and the percent inhibition of AChE, since DFP appears to cause greater inactivation of AChE than does physostigmine over the dose levels tested but has a less pronounced effect on the VER (Fig. 2). The finding that the preferential reduction at low spatial frequencies is dose dependent (Fig. 4) further complicates the expectation of simple explanation, for sensitivity changes may occur in non-cholinergic neurons.

Although we cannot comment on the etiology of the anticholinesterase effect, our results do provide further evidence of the pharmacological distinction between the mechanisms responding to low and high spatial frequencies. In agreement with this distinction, topically applied echothiophate (an organophosphate) primarily reduces low and middle spatial frequency sensitivity in human observers (A.P. Ginsburg; personal communication).

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